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BRIEF COMMUNICATION

Gynandromorph of the squash bee *Eucera* (*Peponapis*) *pruinosa* (Hymenoptera: Apidae: Eucerini) from an agricultural field in western Pennsylvania, USA

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Abstract. Gynandromorphs are anomalous individuals that are genetically chimeric and express both male and female phenotypes. Here, we describe the first record of a mosaic gynandromorph of the squash bee *Eucera* (*Peponapis*) *pruinosa* (Say) from a single specimen collected from western Pennsylvania, United States of America (USA). We discuss the known developmental mechanisms resulting in gynandromorphism and how parasitism or environmental contaminants may instigate these mechanisms in wild bee individuals.

INTRODUCTION

Bees, and most hymenopterans, are haplodiploid insects in which females develop from fertilized diploid eggs and males develop from unfertilized haploid eggs (Heimpel & de Boer, 2008). Female bees store sperm in their spermatheca after mating and determine the sex of their progeny before egg-laying. Genetically, sex is determined by one or multiple sex determination loci where the presence of two different alleles leads to the development of females while the presence of one allele leads to the expression of male phenotypes (Beye *et al.*, 2003). However, abnormalities in sex determination can occur. When the egg and sperm carry the same alleles, fertilized eggs develop as males even though they carry two copies of the genome (Harpur *et al.*, 2013). The frequency of diploid males has been used as an indicator

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of loss of genetic diversity and high levels of inbreeding in bee populations (Zayed & Packer, 2005). Diploid males are also an important marker in conservation genetics studies of bees (Lozier & Zayed, 2016) and have been reported in high frequencies in wasps, bumble bees, and orchid bees (*e.g.*, Schenau & Jha, 2017; Giangarelli *et al.*, 2015; López-Uribe *et al.*, 2007; Zayed *et al.*, 2004).

A less frequent form of abnormality in sex determination is the development of intersex and gynandromorph individuals (Hinojosa-Díaz *et al.*, 2012). Intersex individuals are chromosomally uniform but express ambiguously male and female characters making their identification difficult (Narita *et al.*, 2010). Gynandromorphs are individuals that genetically have both male and female structures and express phenotypes that can clearly be assigned to one sex in different parts of their bodies. Gynandromorphs can be classified into three main categories: bilateral, transverse, or mosaic (Michez *et al.*, 2009). Bilateral gynandromorphs are characterized by a symmetrical distribution of male and female characters along the longitudinal axis of the body. In transverse gynanders, the distribution of male and female characters is found cross-sectional to the body (*e.g.*, the head is of one sex and the body is of the other sex). Mosaic gynandromorphs are defined by the random distribution of male or female characters in different parts of the body and it is the most common type of gynandromorph among bees.

Here, we document the first record of a gynandromorph of the squash bee *Eucera* (*Peponapis*) *pruinosa* (Say). This solitary, ground-nesting bee is a pollen specialist of plants in the genus *Cucurbita* L. (Cucurbitales: Cucurbitaceae), which includes crops such as pumpkins, squash, zucchini, and other gourds (Hurd *et al.*, 1971). Because of the widespread cultivation of domesticated *Cucurbita* spp., *E. pruinosa* recently expanded its range from the xeric areas in northern Mexico and southwestern United States into northern North America (López-Uribe *et al.*, 2016). This bee species is an integral part of the *Cucurbita* agroecosystems in the United States and Canada because of its role as a crop pollinator (*e.g.*, McGrady *et al.*, 2020). Populations of *E. pruinosa* distributed in the extended part of their range, including eastern North America, are exclusively distributed in agricultural areas where they are constantly threatened by local extinction due to tillage, crop rotation, and pesticides (Chan *et al.*, 2019; Ullmann *et al.*, 2016).

MATERIAL AND METHODS

LJJ collected the gynandromorph specimen of *E. pruinosa* from *Cucurbita pepo* L. (cultivar: Apogee) in Butler, Butler Co., Pennsylvania, USA (40°50'35.4"N, 80°02'57.0"W) on 29 July 2019. Before capturing, we observed the individual actively collecting pollen from a male flower of *C. pepo* (LJJ, pers. obs.). We placed the specimen in a perforated falcon tube on ice and brought it back to the laboratory (Pennsylvania State University, University Park, PA), where it was stored in a -20°C freezer.

We photographed external morphological structures using an Olympus SZ61 microscope with and without a 110AL1.5X WD61 lens, and mounted with an Olympus LC30 3.1 megapixel digital color camera (Olympus Corporation, Tokyo, Japan). Digital images were taken manually using the Extended Focal Imaging (EFI) process in the Olympus cellSens Standard V1.18 (Build 16686) Release X64 software (Olympus Corporation, Tokyo, Japan). We viewed image metadata using the OlympusImageJPlugin V2.3.1 (https://imagej.net/OlympusImageJPlugin). We calibrated images for measurement using the "Analyze>Set Scale" feature in ImageJ V1.52q

(Schneider *et al.*, 2012). We measured each morphological feature three times using the "Analyze>Measure" line tool and report mean values in the description. We used Adobe Photoshop CC v21.1.2 to remove shadowing around the bee, using the Paint Brush on Layer Masks and Spot Healing Brush tools. We added scale bars to figures using the "Analyze>Tools>Scale Bar" feature in ImageJ V1.52q (Schneider *et al.*, 2012).

For identification and description, we examined the gynandromorph and conspecific specimens of both sexes collected from three localities: the same locality as the gynander, Cedar Meadows, Lancaster Co., and State College, Centre Co., Pennsylvania, USA. To describe morphological features, we used an AmScope SM-1BSX-64S Professional Binocular Stereo Zoom microscope, equipped with WF10x/20 lenses and an AmScope LED – 64S ring light (AmScope, Irvine, CA). We followed the genus-level taxonomic classification by Dorchin *et al.* (2018) and higher-level classification within Apidae by Bossert *et al.* (2019). SKK confirmed species identifications using Ayala & Griswold's (2012) key. Furthermore, *E. pruinosa* is the only species within the subgenus *Peponapis* Robertson that occurs in Pennsylvania to date (Kilpatrick *et al.*, 2020). We followed the morphological terminology by Michener (2007). Abbreviations used in the morphological description and remarks are as follows: F, flagellomere; T, metasomal tergum; S, metasomal sternum. The gynandromorph specimen is housed at the López-Uribe Laboratory (The Pennsylvania State University, University Park, PA).

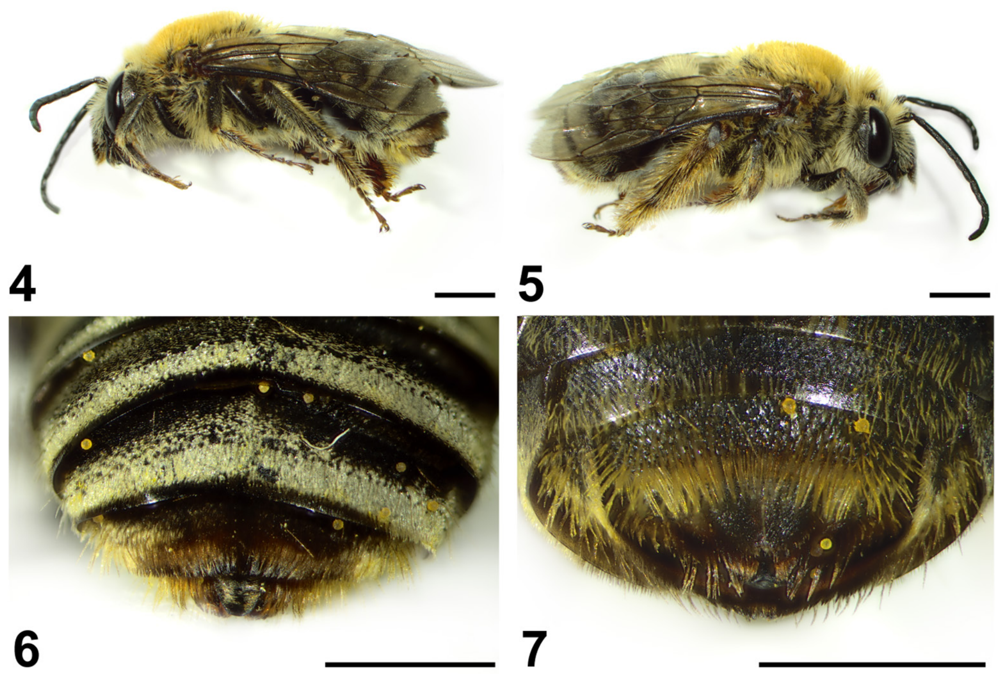
RESULTS

Eucera (*Peponapis*) *pruinosa* (Say)
Gynandromorph

Description: Male and female features are patchily distributed throughout the body (Figs. 1–7). Body length 11.74 mm; integument black unless otherwise noted. Head: width 4.14 mm; length 2.71 mm; clypeus width 1.69 mm; clypeus length 2.72 mm; upper interocular distance 2.44 mm; lower interocular distance 2.54 mm; compound eye length 2.16 mm; eye maximum width 0.75 mm. Left of midline displaying female-specific features; antenna with 10 flagellomeres, clypeus lacking yellow subapical maculation (Fig. 1). F2 longer than F3. Mandible black, with reddish brown apically, and lacking teeth apically. Right of midline displaying male-specific features; antenna with 11 flagellomeres, clypeus with yellow subapical maculation (Fig. 1). Mandible black, with yellow-brown maculation on apical fifth, and lacking apical teeth. Antennae black to dark reddish brown. Mesosoma: intertegular distance 3.30 mm (Fig. 2). Legs: all consistently black to reddish brown. Left legs: all as in males, inner surface of hind basitarsus with longer setae, yet scopa lacking (Figs. 3, 4). Right legs: all as in females, scopa present on hind tibia and basitarsus (Figs. 3, 5). Metasoma: T2 width 5.44 mm; pubescence and structures as in females, consisting of six visible terga and sterna (Figs. 6, 7). T6 with complete pygidial plate (Fig. 6). Sting apparatus normal, including gonostylus, stylus, and lancet, as in females.

Remarks: Notably, F2 is longer than F3, not shorter as in typical females of *E. pruinosa*. As a result, this gynander specimen keys out to *E.* (*P.*) *smithi* (Hurd & Linsley) when following the female couplets in Ayala & Griswold (2012). Additionally, the specimen lacks apical teeth on the right mandible; two teeth are present in most conspecific males. In regards to the apical half of the mandible, the left mandible lacks yellow maculations that most conspecific females possess in varying amounts; the ex-

Figures 1–3. Mosaic gynandromorph of *Eucera* (*Peponapis*) *pruinosa* (Say); scale bars = 2 mm. **1.** Head (in frontal view) showing the distinct bilateral split between the sexes (right half female, left half male). **2.** Dorsal habitus showing male and female antennae, mesosoma, and female-like metasomal features. **3.** Ventral habitus showing patchily distributed female and male features on the head and mesosoma, including the antennae and legs, and female-like metasoma.



Figures 4–7. Mosaic gynandromorph of *Eucera* (*Peponapis*) *pruinosa* (Say); scale bars = 2 mm. **4.** Left lateral habitus showing female antennae and male legs. **5.** Right lateral habitus showing male antennae and female legs. **6.** Metasomal terga (in dorsal view) showing T3–T6; T6 with pygidial plate. **7.** Metasomal sterna (in ventral view) showing typical female features, including a lack of carinae on S6 (present in male *Peponapis*).

tent of this maculation also varies among males. Furthermore, the setae on the left metabasitarsus appear longer, on average, compared to male conspecifics.

DISCUSSION

We describe the first gynandromorph of *E. pruinosa*, which belongs to the mosaic category of gynandromorphs with alternating bilateral female and male tissues distributed along the body. Mosaic gynandromorphs are common gynanders described in bees (Michez *et al*., 2009). Due to the presence of distinct male and female characters with clear boundaries, we interpret the external morphology of this specimen as belonging to a gynandromorph and not an intersex (Narita *et al*., 2010). The latter express intermediate phenotypes between female and male unlike the well-defined structures observed in the specimen here described. When collected, the specimen expressed pollen-collecting behavior typical of a female, and presumably was provisioning a nest. This specimen is only the fourth gynandromorph within the genus *Eucera* and sixth within the tribe Eucerini that we are aware of (Dalla Torre & Friese, 1899; Cockerell, 1906; Masuda, 1940; Urban, 1999), suggesting that gynandromorphism may be uncommon among eucerine bees.

There are a number of hypothesized developmental mechanisms to explain the production of gynandromorphs among insects, including chromosome elimination, post-cleavage fertilization, egg binucleation, and polyspermy (Narita *et al.*, 2010). In bees, sex is determined by a single locus of complementary sex determination (sl-CSD), where females are sl-CSD heterozygous and males are typically sl-CSD hemizygous (Beye *et al.*, 2003). If the chromosome containing the sl-CSD is eliminated in one of the cells at the first somatic division, it will result in the production of both diploid (female) and haploid (male) cells (Bridges *et al.*, 1919; van Wilgenburg *et al.*, 2006). Post-cleavage fertilization has also been posited as a mechanism for the production of gynandromorphs in a number of specimens (Boveri, 1915). By this mechanism, the egg cleaves into two cells before penetration of the sperm, leaving one unfused nucleus and one fused (fertilized). This produces haploid and diploid cells, resulting in sex mosaicism. Binucleate eggs would similarly result in gynandromorphism, as only one nucleus within the cell would be fused with the sperm's nucleus, leaving the second nucleus unfertilized, resulting in partial fertilization (Stern & Sekiguti, 1931). Alternatively, gynandromorphism may arise through polyspermy when more than one spermatozoid enters the egg, leaving one to fuse with the nucleus and the other(s) to remain unfused (Morgan, 1916). When post-cleavage fertilization results in gynandromorphism, it has been argued that the individual is likely to present either a phenotype that is intermediate between both sexes, or that the female phenotype will be dominant (Morgan, 1916). The specimen described here was observed to exhibit a female behavioral phenotype (*e.g.*, pollen collection) shortly before the specimen was captured and has predominantly female physical characters (*e.g.*, female-like metasoma), suggesting post-cleavage fertilization as a possible mechanism for the expressed gynandromorphism.

Many instances of gynandromorphism or intersex individuals in wild conditions have been associated with human-mediated stressors in the environment, including the input of contaminants or pollutants into a system (LeBlanc, 2016). The potential influence of contaminants on gynandromorphism in aquatic vertebrates and arthropods is well documented (Grilo & Rosa, 2017; Devillers, 2020), particularly in Crustacea (Ford, 2012; Dunn et al., 2020). Some insecticides within the phenoxyphenoxy family are known to mimic an important reproductive hormone (methyl farnesoate) involved in the sex determination of crustaceans (Olmstead & LeBlanc, 2007). These insecticides induce gynandromorphism in *Daphnia magna* Straus in laboratory conditions, with rising temperatures posited to increase incidence of gynandromorphism when phenoxyphenoxy insecticides are applied (Olmstead & LeBlanc, 2007). Exposure to herbicides and other human-introduced environmental contaminants, including antifouling agents and estrogenic compounds, has likewise been associated with gynandromorphism in aquatic and semi-aquatic organisms, including molluscs, amphibians, and fish (Huang *et al.*, 2020; Scholz & Klüver, 2009; Chen & Guô, 2008). The role of contaminants in causing gynandromorphism in terrestrial arthropods has been poorly studied, and is limited mostly to the effects of nuclear pollution on Lepidoptera (Dantchenko *et al.*, 1995; Hiyama *et al.*, 2012) and pesticide application on Arachnida (Buczek *et al.*, 2019). However, bee exposure to similar compounds through pesticide application is well documented (Krupke *et al.*, 2012; Gill *et al.*, 2012; Gill & Raine, 2014), suggesting the possibility that pesticides may also induce gynandromorphism in bees which forage in and around agroecosystems.

Parasitism by endoparasitic insects in the order Strepsiptera or by gram-negative bacteria in the genus *Wolbachia* Hertig & Wolbach has also been documented to result in gyanders and intersex individuals within Hymenoptera (Salt, 1927; Werren *et al.*, 2008; Narita, 2010). Strepsiptera parasitism has been found to alter the secondary sexual characteristics of bees in the genus *Andrena* Fabricius, often resulting in female bees exhibiting male sexual characters (Salt, 1927). Species of *Wolbachia* are known to induce parthenogenesis in Hymenoptera (Werren *et al.*, 2008); when females are producing haploid offspring, *Wolbachia* infection can cause failed segregation of the chromosomes during mitotic division resulting in diploidization of the nucleus which creates a female clone (Stouthamer & Kazmer, 1994). Under high temperature conditions however, the interference of *Wolbachia* in the process of mitosis is partially suppressed, postponing diploidization to a later cleavage stage allowing some cells to remain haploid, which results in sex mosaicism (Stouthamer, 1997). The specimen described here was not tested for any parasites or pathogens to avoid tissue damage for preservation purposes. However, superficial examination revealed no indication of the presence of Strepsiptera parasites, which typically protrude between the abdominal segments of the host. Likewise, *Wolbachia* have not been detected in the gut microbiome of *E. pruinosa* collected in Pennsylvania, USA (Shapiro *et al.*, 2019), so we do not expect *Wolbachia* to be present in our specimen.

This finding adds to the growing number of reports of gynandromorphs among bees in recent years (Krichilsky *et al.*, 2020; Villamizar, 2020; Lucia & Gonzalez, 2013; Spring *et al.*, 2015; Ramos & Ruz, 2013; Camargo & Gonçalves, 2013). Detailed descriptions of gynandromorphs are necessary to provide well-documented cases of this phenomenon and how these specimens differ from types of the same species. Because of the low detection of gynanders in wild populations, the underlying developmental mechanisms leading to these phenotypes has not been fully explored for most species. Conducting population surveys in sexually dimorphic insects could capture the frequency of gynandromorphs among wild terrestrial arthropods, and give insight into which factors may contribute to their occurrence (*e.g.*, parasitism, pollution). To investigate the developmental mechanisms resulting in gynandromorphism, there is the need to develop new technological approaches that allow the non-destructive characterization of single cell ploidy levels.

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